developed 6 weeks later (*6*). Blood culture grew *B. longum* and *B. infantis*, which were probiotic strains. Apart from 1 case of sepsis caused by *B. longum* associated with acupuncture in a 19-year-old healthy patient (*7*), we did not find other reports of invasive *Bifidobacterium* spp. infections.

Because neutropenic episodes, even with bowel involvement, are common during treatment for cancer (*8*), no reason to promote therapeutic use of probiotics has been proven. Probiotics can cause substantial bacterial overgrowth when stimulating factors are present. In our opinion, avoiding fecal impaction is crucial for preventing colonic bacterial overgrowth and minimizes the chance that bacteria will translocate and cause invasive infection. Nutritional recommendations for a neutropenic diet for children are still debated. The problem is not probiotic therapy but rather fermented food products to which small amounts of probiotics are added. After we reviewed the literature, we did not find enough data to safely recommend the use of these products in children receiving chemotherapy (*9*). Nevertheless, probiotic therapy is recommended for many immunocompromised patients, such as preterm infants and persons with chronic inflammatory bowel disease (*10*). We believe that this case of *B. breve* sepsis in an oncology patient underscores the invasive potential of probiotics.

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Filovirus RNA in Fruit Bats, China

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To the Editor: Filovirus-associated diseases, particularly those caused by Ebola and Marburg viruses, represent major threats to human health worldwide because they have extremely high death rates and antiviral therapies or vaccines against them are not available (*1*). Members of the family *Filoviridae* are classified into 3 genera: *Marburgvirus*, *Ebolavirus*, and the recently approved *Cuevavirus* (*2*,*3*). Marburg virus (MARV) and Ebola virus (EBOV) were initially isolated in Africa, but other filoviruses have been identified on other continents. The initial *Cuevavirus*, Lloviu virus (LLOV), was identified in Europe (Spain) (*3*), and Ebola-Reston virus has been found in pigs in Asia (the Philippines) (*4*).

Bats are natural reservoirs for filoviruses (*5*). Viral isolation and serologic studies indicate that filovirus infections have occurred in various bat species in central Africa countries (*6*), the Philippines (*7*), China (*8*), and Bangladesh (*9*). However, identification of these viruses in bats has been difficult; although isolates of MARV have been obtained (*6*) and the genome of LLOV has been fully sequenced (*3*), very short sequences of EBOV have been obtained from bats, and only in Africa (*5*). Reports of molecular detection or isolation of filoviruses in bats in Asia are lacking. We conducted a study to investigate the presence of filoviruses in bats in China.

In June 2013, twenty-nine apparently healthy *Rousettus leschenaultia* fruit bats were captured in Yunnan Province, China. All bats were humanely killed, and their intestines, lungs, livers, and brains were collected and subjected to viral metagenomic analysis by a previously described method (*10*). As a result, we obtained and reassembled de novo

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10 million reads into 590,010 contigs. Of these contigs, 3 (129–354 nt) were genetically close to filovirus, corresponding to the nucleoprotein gene of LLOV (74% nt identity), the viral protein 35 gene of Sudan Ebola virus (69% nt identity), and the L gene of Tai Forest Ebola virus (72% nt identity) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/9/15-0260-Techapp1.pdf).

For further screening, we used the longest contig as a template for design of specific seminested primers. Nested degenerate primer pairs were also designed and focused on the most conserved region of the L gene of all currently known filoviruses (online Technical Appendix Table 2). After screening, 2 reverse transcription PCRs of tissues from 1 bat (Bt-DH04) showed positive amplification in specimens from its lung but not from intestine, liver, or brain tissue. Moreover, 5 blind passages in Vero-E6 cells failed to isolate the virus from the lung homogenate. In an attempt to obtain its genomic sequence, 24 primer pairs covering the full genome were further designed by alignment of these contig sequences with the full genomes of representative filoviruses within the 3 genera. All amplifications used ddH_2O as a negative control; positive controls were not available because filoviruses were not available in China. Two fragments of 2,750-nt (F1) and 2,682-nt (F2) were successfully amplified from lung tissue of Bt-DH04; attempts to amplify the remaining regions failed. Alignment with sequences of 26 representative filoviruses of 7 species from 3 genera revealed that F1 covered the 3′ end of the nucleoprotein gene and almost the entire viral protein 35 gene, and that F2 covered the middle region of the L gene, corresponding to nt 1,313–4,085 and nt 12,613–15,302 of the full genome of EBOV (GenBank accession no. HQ613402). The 2 fragment sequences were submitted to Genbank (accession no. KP233864), and the strain has been tentatively named Bt-DH04.

Phylogenetic analysis showed that the Bt-DH04 strain is placed, together with LLOV, at basal position and intermediate between EBOV and MARV (Figure). It is divergent from all known filoviruses, with F1 sharing the highest nucleotide identities (46%–49%) to members of the genus *Ebolavirus*, followed by 44% to LLOV and <40% to MARV (Figure, panel A). The L gene is the most conserved region of filoviruses, and F2 of Bt-DH04 strain shared relatively closer 66%–68% nt identities with members of the genus *Ebolavirus*, followed by 64% with LLOV and ≈60% with MARV (Figure, panel B). This sequence diversity is likely the main factor for unsuccessful amplification of the full genome of Bt-DH04.

Increasing PCR evidence has identified the existence of filoviruses in bats in Africa and Europe (*3*,*5*); however, although serologic studies have shown that filovirus antibodies are prevalent in bats in a few countries in Asia (e.g., the Philippines, Bangladesh and China [*7*–*9*]), filovirus or filovirus RNA have not been reported in bats in Asia. Our results show that the Bt-DH04 strain is likely a novel bat-borne filovirus in Asia and provide evidence that bats in Asia harbor more divergent filoviruses than previously thought.

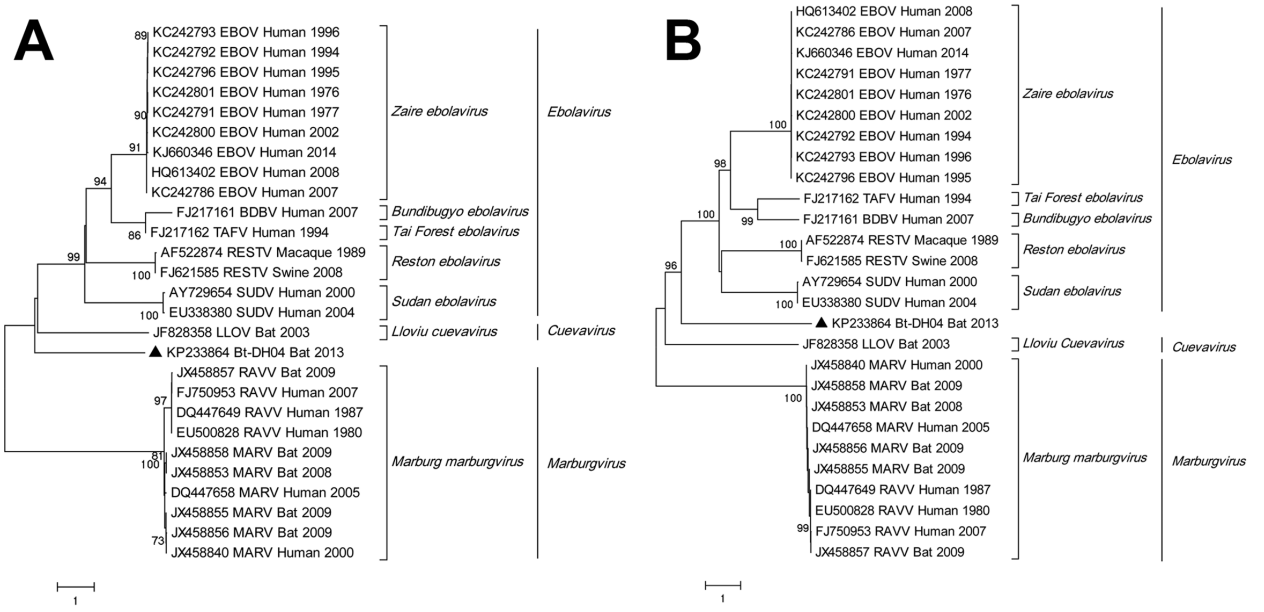

Figure. Phylogenetic analysis of 2 fragments of filovirus Bt-DH04 and other filoviruses. Full genomes of representatives from the family *Filoviridae* were trimmed and aligned with F1 (partial nucleoprotein/viral protein 35 gene, panel A) and F2 (middle L gene, panel B) of filovirus strain Bt-DH04 by using ClustalW version 2.0 (http://www.clustal.org), then phylogenetically analyzed by using MEGA6 (http://www.megasoftware.net) by the maximum-likelihood method, resulting in a bootstrap testing value of 1,000. Sequences are listed by their GenBank accession numbers, followed by the virus name, host, and collection time. Triangles identify the novel filovirus strain Bt-DH04 (China). Scale bars indicate nucleotide substitutions per site.

Fruit bats in the genus *Rousettus* are widely distributed throughout Southeast Asia, South China, and the entire Indian subcontinent and have had positive serologic results for Ebola viruses in these regions (*7*–*9*), indicating that these bats play a role in the circulation of filoviruses in Asia. The possibility of new emerging filovirus-associated diseases in the continent emphasizes the need for further investigation of these animals.

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Increase in Lymphadenitis Cases after Shift in BCG Vaccine Strain

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To the Editor: Bacillus Calmette-Guérin (BCG) vaccine is one of the most commonly used vaccines for tuberculosis (TB) worldwide (*1*). The original BCG strain was developed in 1921. Numerous strains have since been developed, and 5 strains, including Danish SSI 1331 (Statens Serum Institute, Copenhagen, Denmark), account for >90% of BCG vaccine used. Each strain has unique characteristics and a different reactogenicity profile (*2*). The most common severe adverse events related to BCG vaccination are nonsuppurative and suppurative lymphadenitis.

In the country of Georgia, BCG vaccine is administered routinely to infants (estimated coverage 96%); the National Center for Disease Control and Public Health receives its vaccine supply from the United Nations Children's Fund and is responsible for countrywide distribution. Before 2012, Russian BCG-I (Bulbio, Sofia, Bulgaria) and Danish SSI 1331 strains were used (≈50% each). Shortly after a change to exclusive use of the Danish 1331 strain during 2012–2013, an increasing number of BCG-related lymphadenitis cases were reported to the National Center for Tuberculosis and Lung Diseases (NCTLD). We aimed to quantify the increase in cases of BCG lymphadenitis and to evaluate clinical management of the cases. The Institutional Review Boards of Emory University (Atlanta, GA, USA) and the National Center for Disease Control and Public Health approved the study.

Medical chart abstraction was conducted for all infants with BCG lymphadenitis either reported to the NCTLD or found by inquiry of pediatricians at the largest children's hospital in the country during January 2012–July 2013. We used national surveillance data to obtain the number of live-born infants.

BCG vaccine is given intradermally over the deltoid muscle on the left arm to infants within 5 days after birth at the maternity hospital. BCG lymphadenitis was clinically

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Filovirus RNA in Fruit Bats, China

Technical Appendix

Sample collection, preparation, and outcomes of testing for filoviral RNA in fruit bats, China

In June 2013, 29 apparently healthy *Rousettus leschenaulti* bats were captured by using a bird net in a longan orchard in Yunnan province of China. Living bats were euthanized by intravenous injection of potassium chloride at the local Center for Animal Diseases Control and Prevention. We intended to focus on encephalitis-related viruses in brains, respiratory tract-borne viruses in lungs, and viruses in digestive system, therefore the brains, lungs, livers and intestines of each bat were collected separately into cryotubes and immediately frozen in a liquid nitrogen tank prior to transportation to the laboratory, where they were stored at –80°C.

Bat samples were prepared for metagenomic analysis as described by He et al. (*1*). The intestines, lungs, livers, and brains of 29 bats were pooled together and homogenized in SM buffer (1:10 [w/v]; 50 mM Tris, 10 mM MgSO4, 0.1 M NaCl, pH7.5). The homogenized samples were centrifuged at 8,000×g at 4□C for 30 min to remove cell debris and foreign materials, and the supernatants were immediately filtered through 0.45-μm and 0.22-μm filters (Millipore). Host genomes and other free nucleic acids were eliminated by digestion of nuclease mixture containing DNase (Ambion), Benzonase Nuclease (Novagen) and RNase I (Fermentas).

The viral RNAs were then extracted immediately using QIAamp® Viral RNA Mini Kit (Qiagen) according to the manufacturer's protocol. Total viral RNAs were dissolved in RNase-free H2O and used immediately for the following reverse transcription with SuperScript III reverse transcriptase (Invitrogen) using anchored random primers according to the manufacturer's protocol. To synthesize dscDNA, a Klenow fragment (New England Biolabs)

was added to the cDNA mixture, and incubated at 37°C for 60 min. After inactivation of the enzyme, phosphates and free single-stranded bases in the dscDNA reaction was removed using shrimp alkaline phosphatase and exonuclease I (TaKaRa).

To obtain sufficient viral nucleic acid, single primer amplification was employed to amplify the dscDNA with the Accuprime Taq DNA Polymerase System (Invitrogen) according to the manufacturer's protocol. Briefly, a 50 μl reaction system containing 10 μl of the above dscDNA mixture, random primers (20 mM), 10×Accuprime buffer I, and Taq DNA Polymerase (1 U) was denatured at 94°C for 2 min, followed by 40 cycles of 94°C denaturing for 30 s, 54°C annealing for 30 s, 68°C extending for 1 min with final 68°C extension for 8 min. The PCR products were then purified using the QIAquick PCR Purification Kit (Qiagen) and dissolved in 50 μl TE buffer (100 mM Tris-HCl, 10 mM EDTA, pH8.0). The purified PCR products were pooled together and then subjected to HiSeq 2000 (Solexa) sequencing in 1 lane by the Beijing Genome Institute (BGI).

To screening filovirus in these samples, the longest contig generated by viral metagenomic analysis was used as a template for design of specific semi-nested primers (Filo-F/Filo-in-F/Filo-R). Nested degenerate primer pairs (FV-F1/FV-R1, FV-F2/FV-R2) were also designed targeting the most conserved region of the L gene of all currently known filoviruses. Viral RNA of each tissue was extracted as above description; cDNA was synthesized by random hexamers and SuperScript III reverse transcriptase (Invitrogen). The 2 PCR methods were conducted by the same Master PCR Mix (Tiangen) and program: denaturing at 94°C for 2 min, followed by 30–35 cycles of 94°C denaturing for 30 s, 54□C annealing for 30 s, 72°C extending for 40 s with final 72°C extension for 5 min. PCR products with expected size were directly sequenced by an ABI 3730 DNA Analyzer (Invitrogen).

The positive sample obtained by above RT-PCR screening was used for full genome amplification. A total of 2 dozen degenerate primer pairs covering the full genome were further

designed by alignment of these contigs with the full genomes of representative filoviruses within the 3 genera. The positive cDNA and LA Taq DNA polymerase (TaKaRa) were used to amplify full genome according to the manufacturer's protocol. The reaction system was denatured at 94°C for 2 min, followed by 40 cycles of 94°C denaturing for 30 s, 54-57°C annealing for 30 s, 72°C extending for 1–3 min with final 72 □C extension for 7 min. Negative control was ddH2O, but positive control was not considered due to filoviruses not available in China as well as a lack of corresponding biosafety facility. Positive amplicons were purified and ligated into the pMD18-T vector (TaKaRa), used to transfect TOP10 chemically competent Escherichia coli (Tiangen), and then sequenced by an ABI 3730 DNA Analyzer (Invitrogen). Of each amplicon, 5 clones were sequenced.

Technical Appendix Table 1. Sequences of contigs generated by viral metagenomic analysis and their identities to filoviruses*

Contig	bp	Sequence (5′→3′)	Gene	Nucleotide identity	Virus (Genbank Accession no./location)
1	207	AATAATCTGGAACATGGATTGTATCCTCAGTTATCAGCTAT AGCAATAGGTATTGCAACTGCACATGGCAGCACACTTGGA GGTGTTAATGTAGGTGAACAATTTCAACCATTGCGTGAAG CTGCAACAGAGGCAGAAAAGCAGCTACAACGGTACACAG AAATAAAGGAATTGGACCAGCAAGGTTTAGATGATCAAGA ACGTAAG	NP	74%	Lloviu virus (JF828359/1,395-1,601)
2	354	GTGAAATGCTGTCCCGAACAATTGAAAGATGCTCATGAGA ATTTGACTCGTGTTAATTCATTAACTGAAAAAACTTTTGCTA AACCTAGTTTTACAGCTAAAGAACTTAGAGATATGATATAT GATCATCTTCCAGGATATGAGACAGCATTTCACCAATTAAC ACAGGTAATATGCAAGATTGCAAAAGATGAGGGTCAATTG GAGCAAGTTCATACAGAATTTCAATCCTCCTTAGCTGAAG GTGATTCTCCACAAAGTGCATTAATACAATTGACTAAACGC ATGACTATTTTCGACGGAAGATCACCTCCACTGATTCATAT AAACACACGAGCAGCCTACGACGACGAT	VP35	69%	Sudan ebolavirus (KC24278/3,668-4,022)
3	129	TTCTCTGGATTCAAAAAGGACACAGACATTGGGCTATTAG CACTCAAAAAGCCATTAGATTATAGTACAATTGTTGTCACC TTATCAATACCCCAAGTCTTAGGGGGATTATCATTTTTGAA TCCAGAG	L	72%	Tai Forest ebolavirus (FJ217162/14,191-14,319)

Contig	bp	Sequence (5′→3′)	Gene	Nucleotide identity	Virus (Genbank Accession no./location)

*NP, nucleoprotein; VP, viral protein.

Technical Appendix Table 2. Primers used in this study. Primers Filo-F, Filo-in-F, Filo-R, FV-F1, FV-R1, FV-F2 and FV-R2 are used to screen filovirus in samples, the rest are used to amplify full genome. The locations of primers refer to the position on the genome of EBOV (HQ613402).

Primer	Sequence (5′→3′)	Polarity	Location
Filo-F	TGATATATGATCATCTTCCAGG	+	3,727-3,748
Filo-in-F	GCATTTCACCAATTAACACAGG	+	3,759-3,780
Filo-R	TTTATATGAATCAGTGGAGGTG	–	3,920-3,941
FV-F1	GCMTTYCCIAGYAAYATGATGG	+	13,261-13,282
FV-R1	GTDATRCAYTGRTTRTCHCCCAT	–	13,713-13,739
FV-F2	TDCAYCARGCITCDTGGCAYC	+	13,316-13,336
FV-R2	GIGCACADGADATRCWIGTCC	–	13,646-13,666
F1	ATCTGGAACATGGMYTGTATCC	+	1,308-1,329
F2	GGAACATGGMYTGTATCCTCAG	+	1,312-1,333
R1	GYTTYTCWGCCTCTGTTGCAGC	–	1,424-1,445
F3	ACTGCTGCAGCTACTGAAGCTTACTGG	+	3,510-3,536
F4	AAAGATGCTCATGAGAATTTGACTCG	+	3,780-3,805
F5	TCCTTAGCTGAAGGTGATTCTCC	+	3,843-3,865
F6	CCAGGATATGGGACAGCATTTCACC	+	3,889-3,913
F7	AAGATTGCAAAAGATGAGGGTCAATTGG	+	3,933-3,960
F8	CCTCCTTAGCTGAAGGTGATTCTCC	+	3,986-4,010
F9	CARAAVWYVTAYAGYTTTGAYTCAAC	+	4,704-4,729
R2	GCDGMNGYNGTTGARTCAAARC	–	4,717-4,738
R3	GGNACACCDGHHCKRAADSCCCA	–	6,213-6,235
F10	YCCHMGNTGYCGBTATGTICAC	+	6,353-6,374
R4	TGTGNACATAVCGRCANCKDGG	–	6,354-6,375
F11	CRGACACACAAAAASAADRRA	+	7,237-7,257
F12	TGGACDGGNTGGMRRCARTGG	+	7,889-7,909
R5	AYCCAYTGYYKCCANCCHGTCC	–	7,890-7,911
F13	VVDTTYGARGCHGCVYTRTGGC	+	9,088-9,109
F14	GCHGCVYTRTGGCARSRDTGGG	+	9,097-9,118
F15	WGCVYTRTGGCARCADTGGG	+	9,099-9,118
R6	GAYYSYCKRTCCCAHTGYTGCC	–	9,107-9,128
R7	GTCAKBGHCCAKGCWGGDGC	–	10,459-10,478
F16	GARTAACTAYGARGAAKATTAA	+	11,410-11,431

Primer	Sequence (5′→3′)	Polarity	Location
F17	CCHATHGTNYTVGAYCARTGTG	+	11,545-11,566
F18	CCHATWRTBYTVGAYCARTGTGA	+	11,545-11,567
F19	TNCARAARCAYTGGGGBCAYCC	+	12,611-12,632
R8	TAGTTCATTGTGGAGTACAGGATGCC	–	12,627-12,652
R9	GTAATTTCAACTTTGTGGCATG	–	12,673-12,694
R10	CACTGGTACTAGTTCTATTGTGATGCC	–	13,331-13,357
R11	CTAGTGAACTCATATCTAAAGGC	–	13,414-13,436
R12	AATGGTCTAGTGAACTCATATCTAAAGGC	–	13,414-13,442
R13	GGTCTAGTGAACTCATATCTAAAGGC	–	13,415-13,440
R14	TTTGGCTTGTTAGACAGTGAGGTGG	–	13,543-13,567
R15	AATTTGAGCACATGATATGCTAGTCC	–	13,647-13,672
F20	GATTAACATGGGCAACGCAAGG	+	15,173-15,194
F21	GTGTTAATTTGGAGGTTGAGG	+	15,240-15,260
F22	TGTTACCAGTACATTATTCAGG	+	15,272-15,293
R16	GWRTTRCTCATNCKRTTBGCCAT	–	15,340-15,362
F23	GCNAGYAAYTTYTTYCAYGCDTC	+	16,393-16,415
R17	TCRTMHARYTTRTAATGCATKGA	–	16,933-16,955
R18	CYTCRTCNARYTTRTARTGCATKG	–	16,934-16,957
R19	GCHARKGAHGCRTGRAARAARTTRC	–	16,937-16,421
R20	TYYTYDGTNGTYTCWGCATCCAT	–	17,275-17,297
R21	WRTTYYCWRTHGTYTCWGCATCC	–	17,277-17,299

Reference

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